Determination and Interpretation of Alkaline Phosphatase Activity in Butter

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(Final Report)

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CONTENTS

I. INTRODUCTION

A study was conducted to investigate the present methods and standards for controlling the pasteurization history of butter. The present AP methods for butter were developed based on limited work done in the 1930's and 1940's and do not account for the possibility of AP reactivation, a common phenomenon in dairy foods today. Although much research has been done on the subject of AP reactivation little of this work has been performed on butter. It is questioned therefore whether the developed methods and standards are meaningful for analysis of butters.

This project involved 2 facets -- a study on butters made in-house and an investigation of commercial butters. The applicability of the AOAC Methods for AP analyses (residual, reactivated, and microbial) was considered as was the present standard of acceptance for butter (<2 units of activity). In addition, effects of pasteurization and storage parameters on AP reactivation were studied. AP analyses were done with AOAC and Rutgers methods. The following report is a summary and discussion of the results.

II. SHELF LIFE STUDY

A. Introduction

B. Results

1. Cream

2. Butter

C. Discussion

D. Summary and Conclusions

A.INTRODUCTION

Butters were manufactured in-house and stored at 40F, 0F, and -20F. Analyses were done every three months for 18 months (except for the 9 month assay). Tests performed were as follows:

Cream:

Alkaline phosphatase activity

Fat content

Microbial plate counts (SPC, Psychrotroph, and Coliform)

Butters:

Alkaline phosphatase activity

Microbial plate counts (SPC, Psychrotroph, and Coliform)

Proximate analysis (at 0 and 18 months)

Aqueous Extracts of Butters:

Alkaline phosphatase activity (at 12, 15, and 18 months)

The specific experimental procedures and results were presented in previous progress reports. The following is a summary and discussion of the observations.

B.RESULTS

1.Cream:

a)AP Activity: The AP values will be discussed in the sections covering the AP activities of the respective butters.

b)Fat Content: Two lots of raw cream were obtained from Welsh Farms. The first lot tested 31% fat. Only the raw cream butter was made from this cream. The five remaining butters were made from a second lot of cream which tested 36% fat.

c)Microbial Plate Counts: Except for some 40F stored butters total microbial and psychrotroph counts were low. No coliforms were observed in any samples. Specific results were included in Progress Report #2.

2.Butter:

a)AP Activity:

(1)165/30min.: Figure 1 shows the activities of the butters made from vat pasteurized cream. Average values for residual AP activities are plotted versus time of storage for all 3 storage temperatures. It is evident that no activity developed in any of the samples over the course of the study. Rutgers Method

analyses and AP determinations on aqueous extracts confirmed these observations. The absence of AP activity in these samples is consistent with literature; prolonged heating permanently inactivates AP.

The AP activity of the vat pasteurized cream before churning was .44 units (AOAC Method). This slight activity was attributable to reactivation.

(2)185/24sec.: Figure 2 shows the results for the butter made from the 185F HTST cream. Average AP activities for samples at the 3 storage temperatures are plotted versus months of storage.

During the shelf life study activities for all samples were less than 2 units, the present standard value of acceptance for butter. A gradual increase in activity is observed after 6 months of storage. No significant differences in AP activity are evident for samples at the 3 different storage temperatures.

The above observations were confirmed by the Rutgers Method on both whole butters and their aqueous extracts as well as the AOAC Method applied to aqueous extracts. The detected activities were not attributable to either microbial or reactivated AP based on the AOAC methods.

The 185/24sec. cream had no activity before churning.

(3)195/24sec.: Figure 3 shows the results for the butter samples made from 195F HTST cream.

As in the previous 2 samples all activities were less than 2 units. An increase in activity is observed after 6 months of storage. No significant differences are apparent between the samples at the three holding temperatures, although an atypical

FIGURE 1.

AP Activity in stored butter --165 F/30 min.--

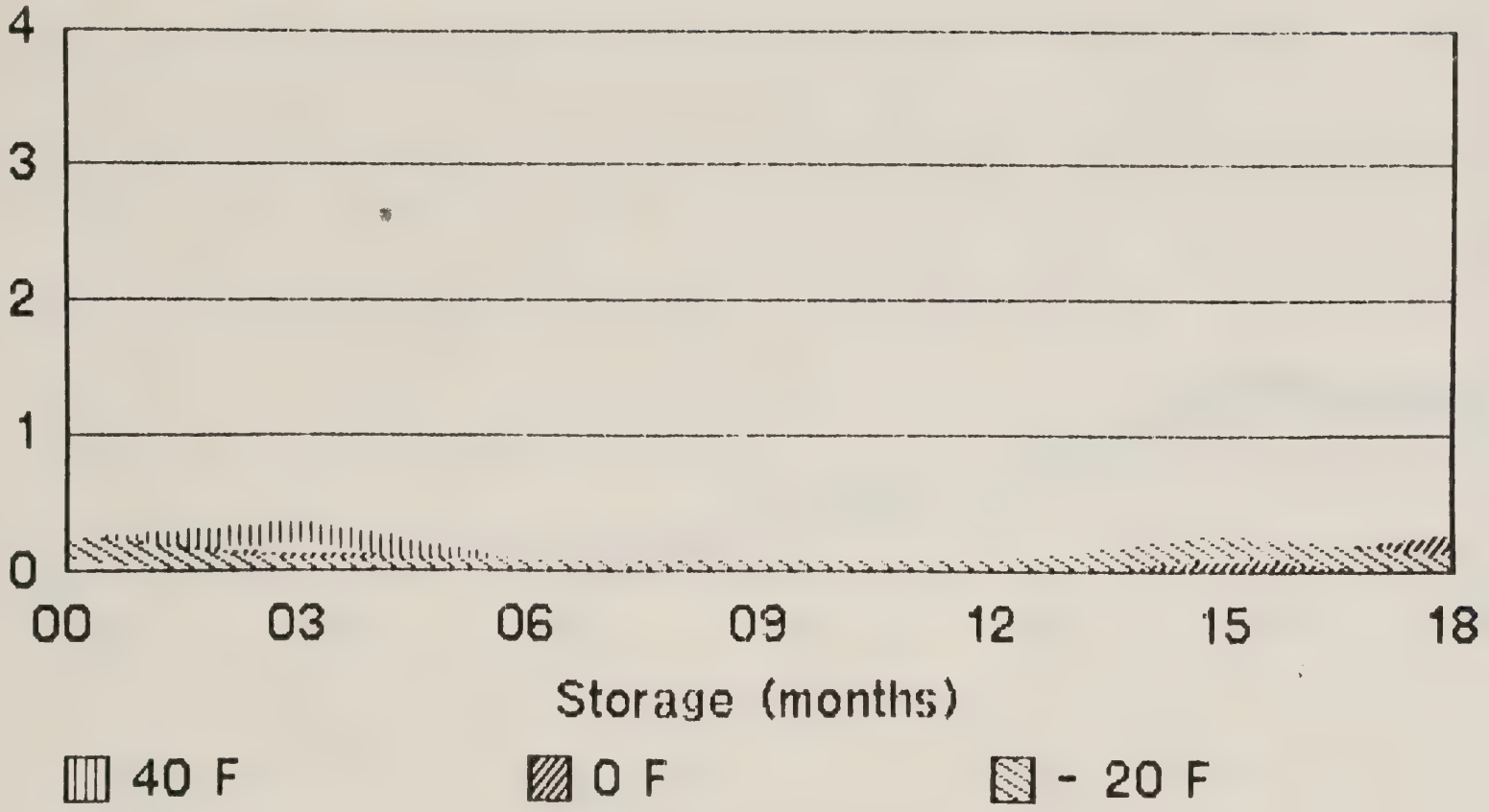
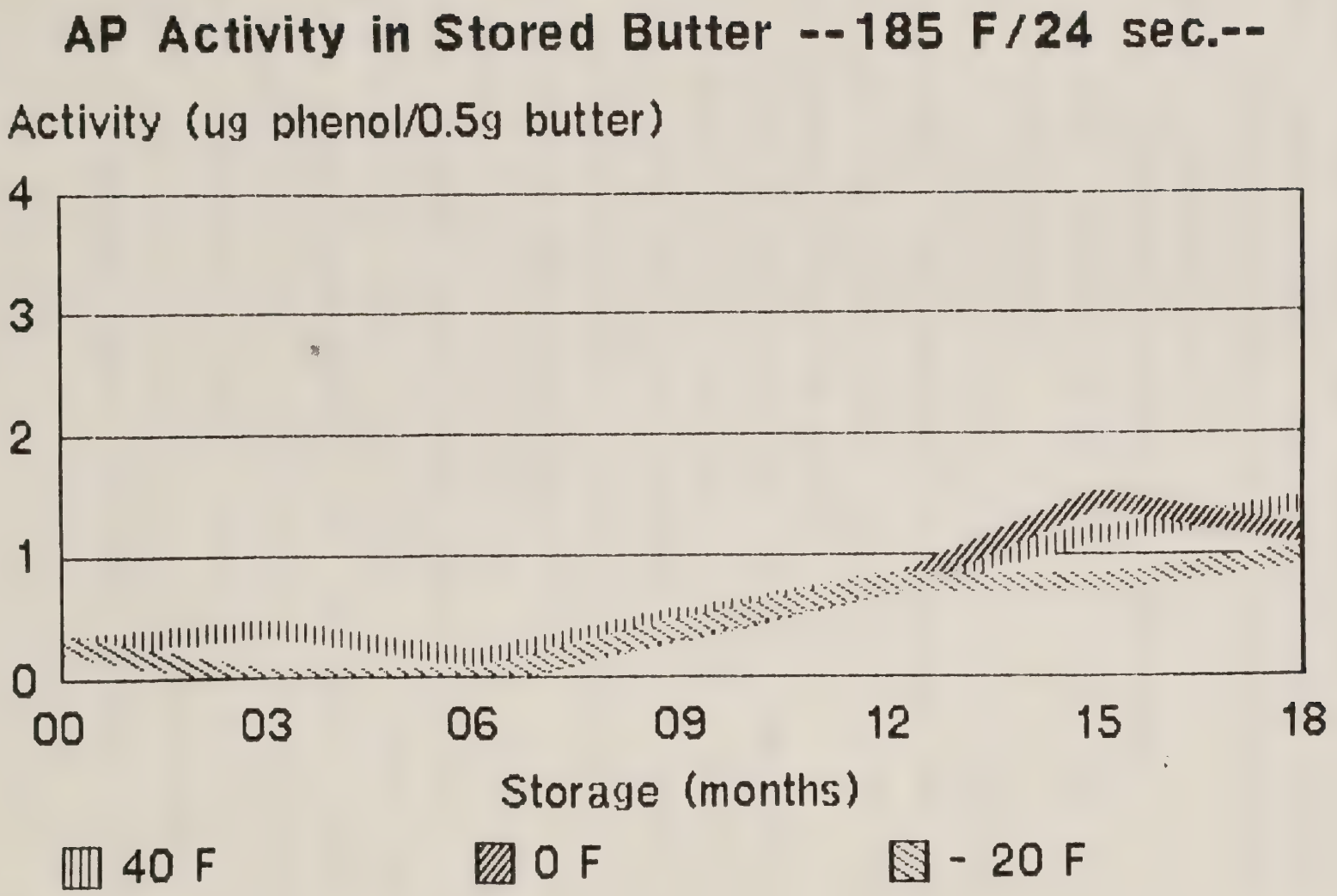


FIGURE 2.


AP Activity in Stored Butter --185 F/24 sec.--
Activity (ug phenol/0.5g butter)
4
3
2
1
0
00
03
06
09
12
15
18
Storage (months)
40 F
0 F
- 20 F

peak in activity is seen in the 0F sample after 15 months of storage. The aqueous phase activity of this sample is very similar to the aqueous phase activities of the 40F and -20F 15 month samples. Thus, this peak could be due to error of measurement or inhomogeneiety of sample. This demonstrates how the aqueous phase analysis can be used to confirm AP activity determinations on whole butters.

Tests for microbial and reactivated AP were negative.

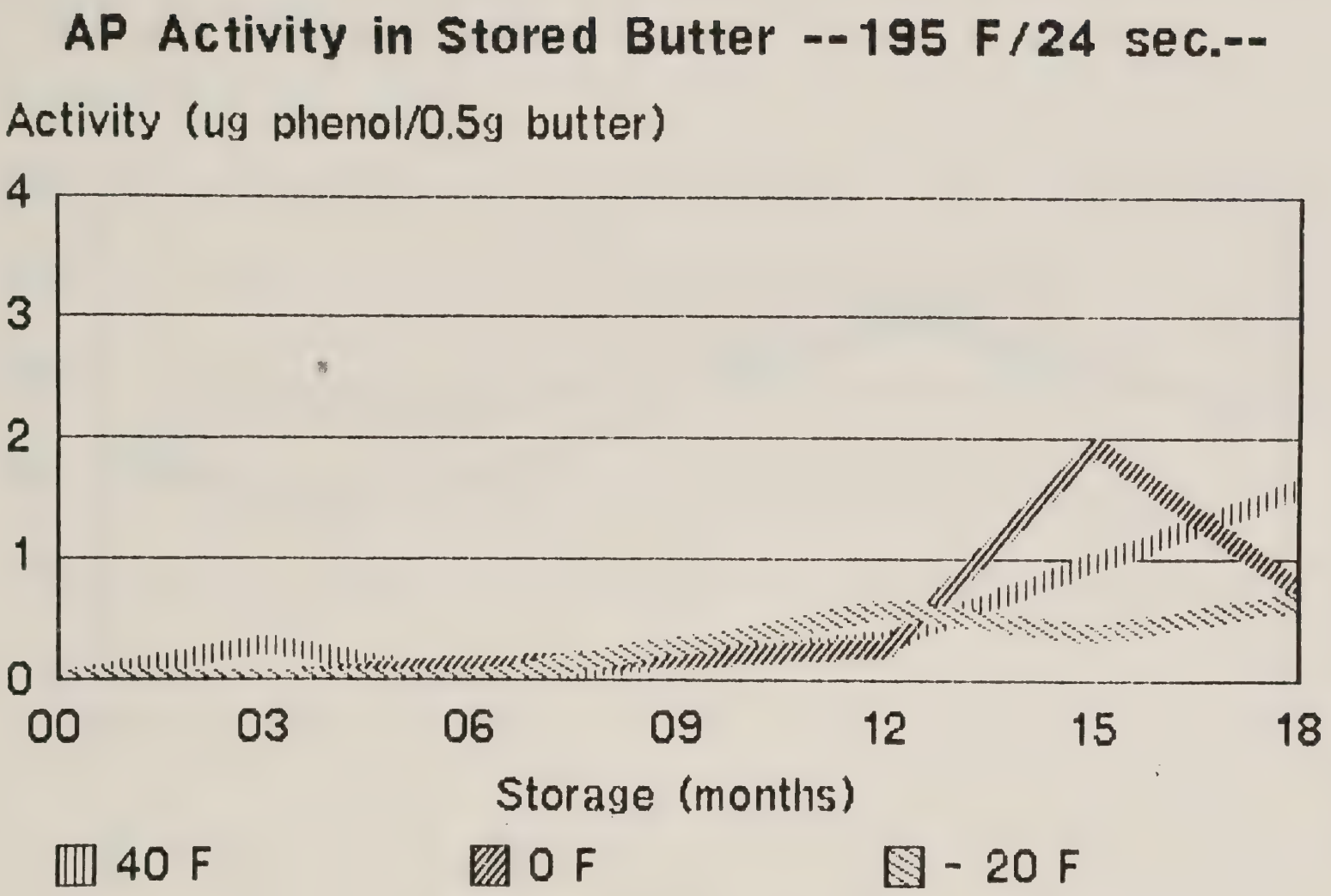
The 195/24sec. cream had an activity of 1.29 units. This was shown to be reactivated AP.

(4)145/24sec.: Figure 4 shows results for the butter made from the 145 HTST cream. This represents the first sample made from underpasteurized cream; consequently, the activities are much greater than those of the previous samples.

A plot of activity versus time reveals a peak after 12 months of storage. At this point the activity of the butters approximately doubled the initial activity at 0 months of storage. Differences in activity between the samples at the three storage temperatures were not significant.

The Rutgers Method determinations on both whole butter samples and their aqueous phases confirm the high activities. In all cases results were greater than 5 std., the highest visual standard used in the Rutgers Method. The AOAC Method was not used on the aqueous extracts of these or the following 2 butters made from the mixed and raw creams. Measuring the very high activities of these aqueous extracts would have necessitated the use of several dilution steps, additional amounts of BQC

FIGURE 3.


AP Activity in Stored Butter --195 F/24 sec.--
Activity (ug phenol/0.5g butter)
4
3
2
1
0
00
03
06
09
12
15
18
Storage (months)
40 F
0 F
- 20 F

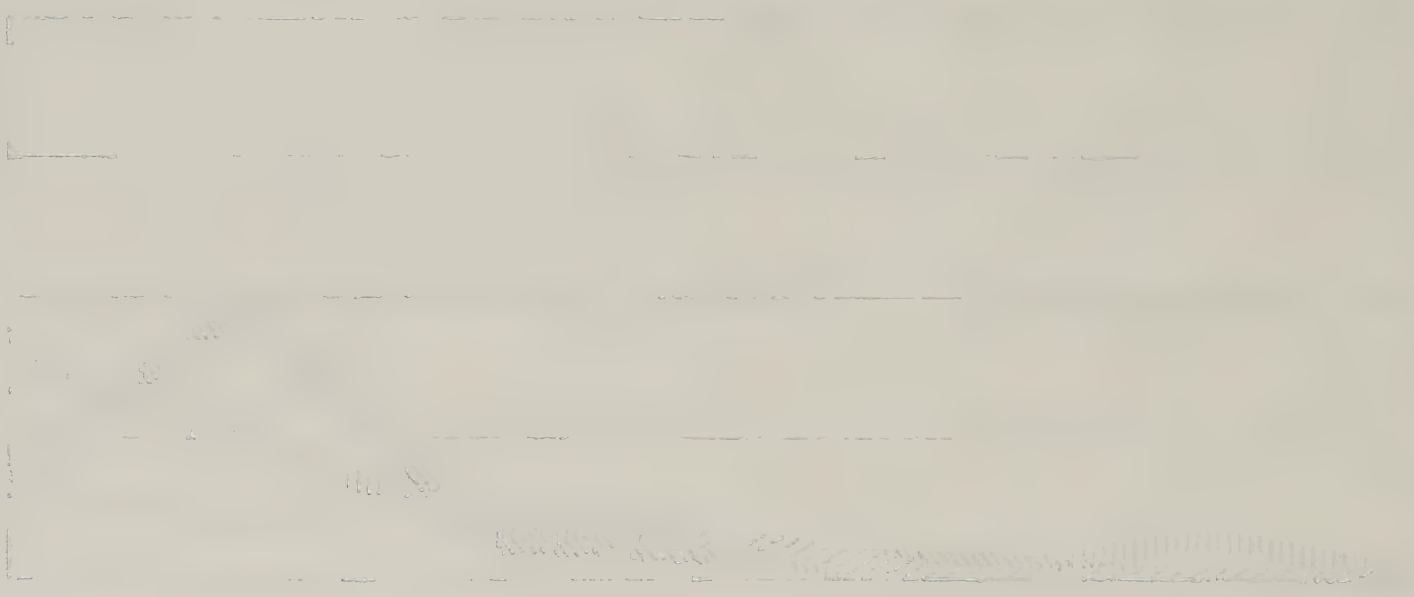
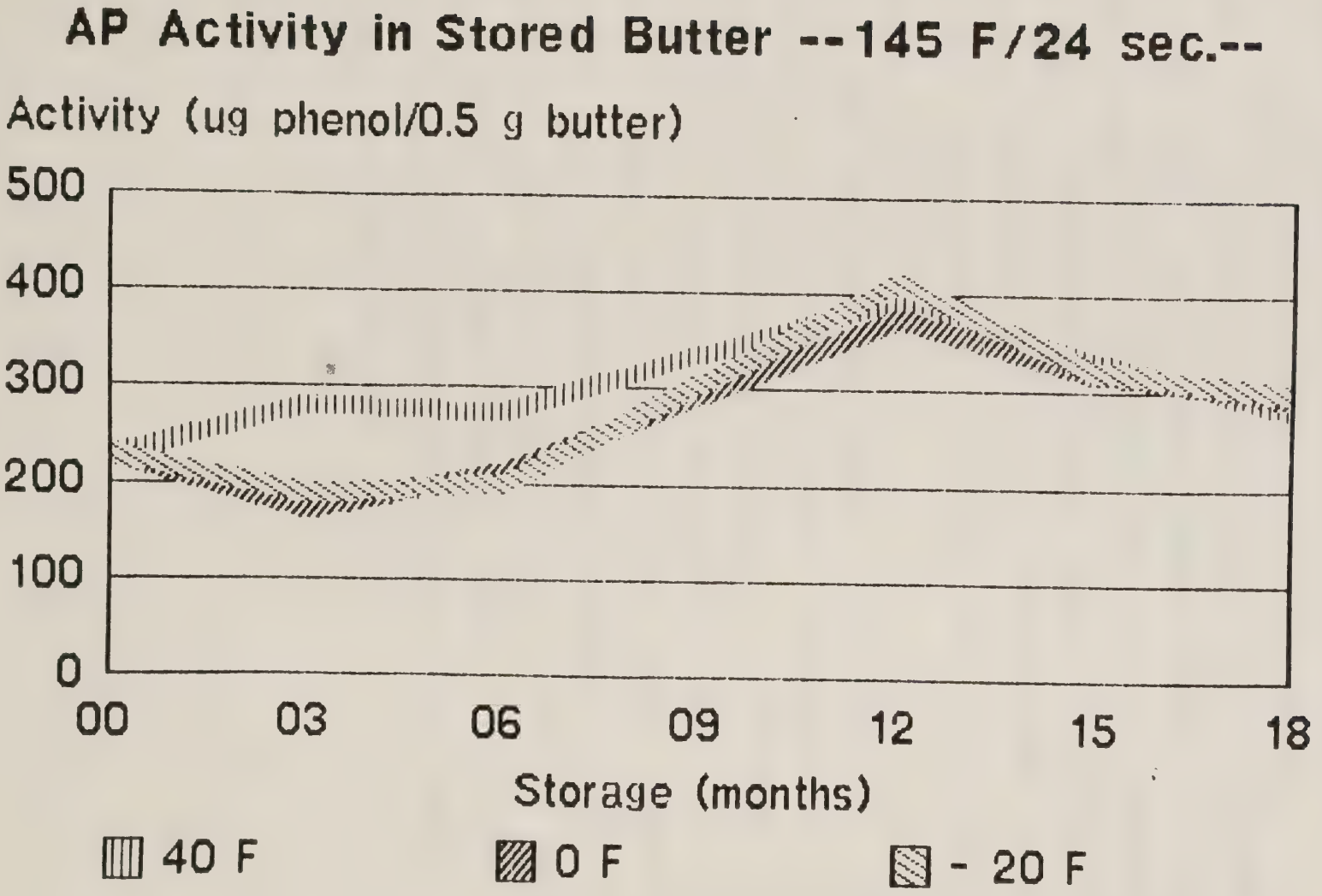


FIGURE 4.


AP Activity in Stored Butter --145 F/24 sec.--
Activity (ug phenol/0.5 g butter)
500
400
300
200
100
0
00
03
06
09
12
15
18
Storage (months)
40 F
0 F
- 20 F

indicator, and an increased color development time. These alterations in procedure would have resulted in less accurate measurements and therefore the aqueous phase assay was not deemed necessary for these samples.

Results for microbial and reactivated AP were negative.

The 145/24sec. cream had an activity of 590 units.

(5)Mixed: The butter from mixed cream (Figure 5) showed similar trends as in the 145/24sec. sample, however , at lower activities. AP activity doubled after 12 months of storage and no significant differences are evident between the samples held at the three temperatures. These results were confirmed by the Rutgers Method determinations. Activities were not attributable to microbial or reactivated AP.

The mixed cream was not tested for AP activity. The activities of its constituent creams are reported in the respective sections (185F/24sec., 195F24sec., and 145F24sec.).

(6)Raw: The raw cream butter showed similar trends as in the previous 2 butters from underpasteurized samples (Figure 6). Activities in the raw cream butter were the greatest of the 6 in-house samples. The activities doubled after 12 months of storage and were similar for the different storage temperatures. No microbial or reactivated AP were detected by the AOAC Methods.

The raw cream had an activity of 750 units.

FIGURE 5.

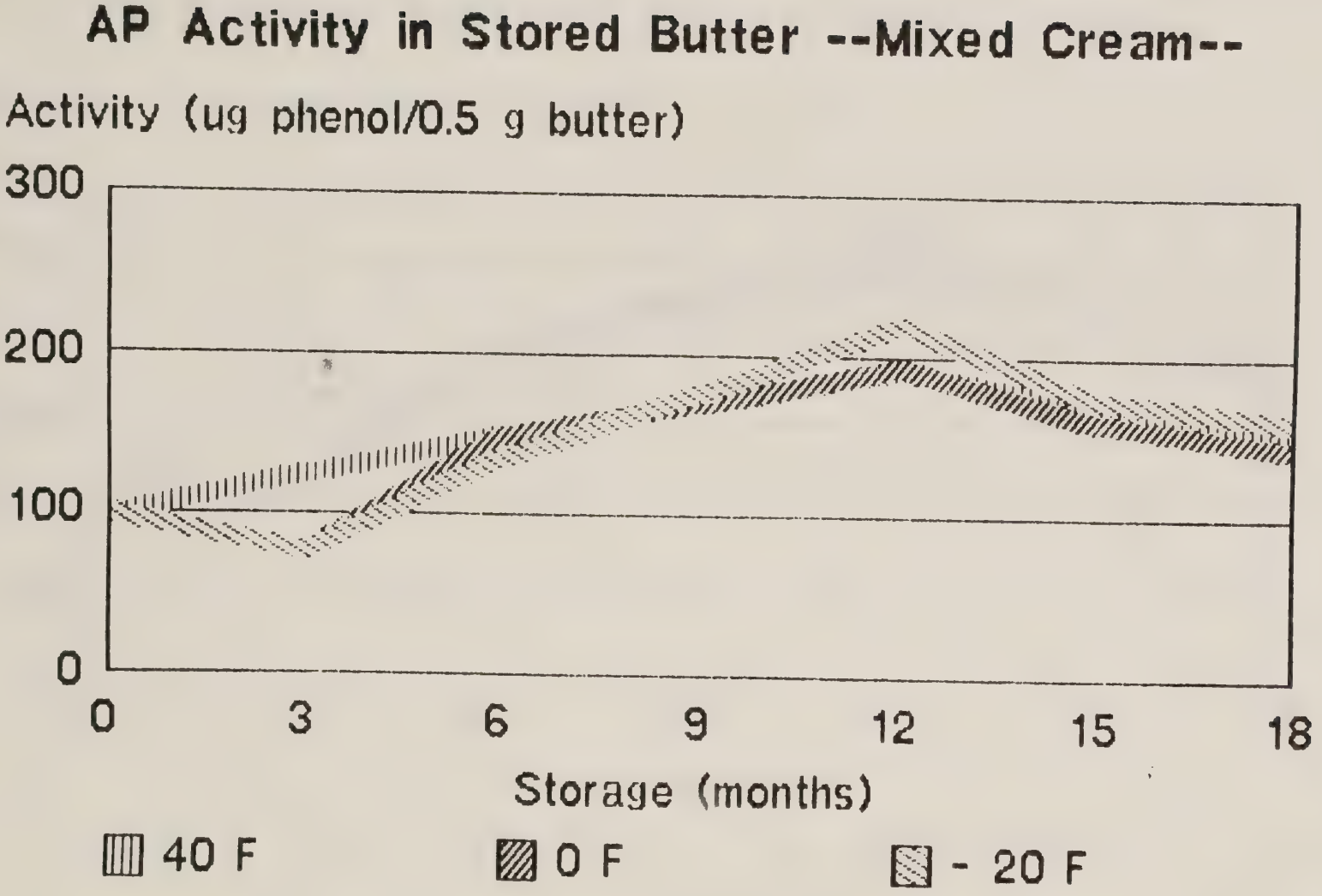
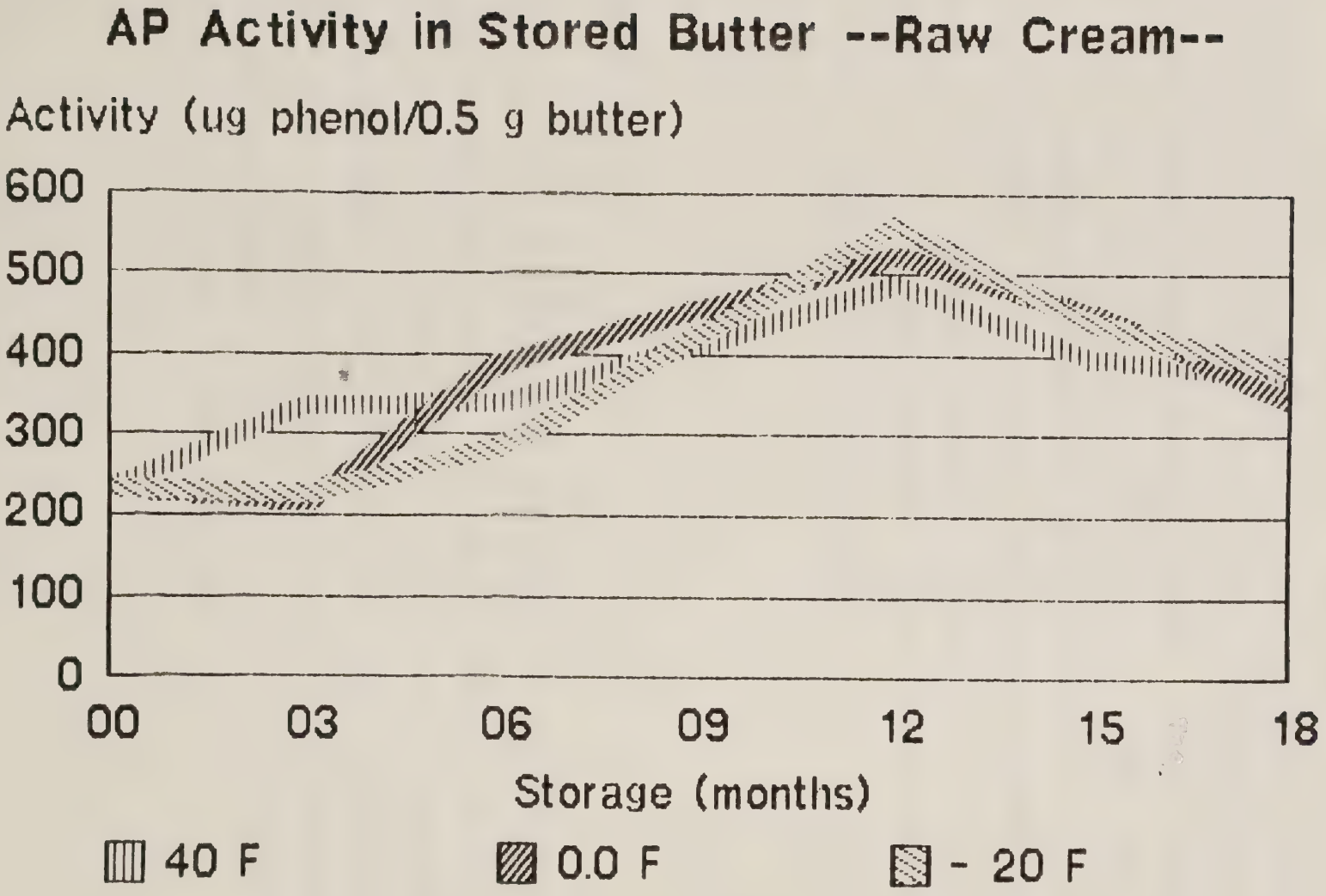

AP Activity in Stored Butter --Mixed Cream--
Activity (ug phenol/0.5 g butter)
300
200
100
0
0
3
6
9
12
15
18
Storage (months)
40 F
0 F
- 20 F

FIGURE 6.


AP Activity in Stored Butter --Raw Cream--
Activity (ug phenol/0.5 g butter)
600
500
400
300
200
100
0
00
03
06
09
12
15
18
Storage (months)
40 F
0.0 F
- 20 F

b)Microbial Plate Counts: Based on the reported results for total plate, psychrotroph, and coliform counts the presence of microbial AP is improbable. This confirms the negative results for microbial AP determinations on the butter samples.

c)Proximate Analyses: Proximate analyses were performed at the beginning and end of the study. As discussed in Progress Report #6 deviations in butter composition were noticed. These changes were erratic, as some samples changed more than others. Typically, any changes in moisture or fat content were within 1% of the original values at 0 months. Salt and curd showed even less variation. The greatest changes in pH were observed in the 40F stored butters. No correlation can be made between the changes in AP activities and butter composition.

C.DISCUSSION

1.General: The creams from properly pasteurized creams all showed less than 2 units of activity. The vat pasteurized sample had no activity over the 18 month study. A slight increase in activity was measured in the 185F and 195F HTST cream butters which was not attributable to either microbial or reactivated AP. No significant differences in activity were noticed in samples held at the three different storage temperatures.

The butters from underpasteurized creams had significantly greater AP activities than the samples described above. In each of these 3 samples the activities doubled during storage. This doubling of activity is an interesting but unexpected observation. Typically, an increase in AP activity has been associated with dairy foods which have received a high degree of heat treatment. These last three samples were made from creams which received little or no pasteurization. Perhaps this indicates that the mechanism of AP reactivation or rather increased AP activity during storage is different for butter than for fluid milk product.

AP activities in the last three samples could not be ascribed to microbial or reactivated AP. Differences in activity for samples held at the various storage temperatures were insignificant.

2.AOAC Method: The present AOAC Method proved to be adequate for determining AP activities in butter. However, the sensitivity of the test on butters with low activities (0-2 units) leaves room for interpretation. A typical deviation between the average value and a high or low value in such butters is about .25 units (refer to previous reports). In some samples this could represent a relative error of 30-40%. For a sample with a borderline AP activity such an error is unacceptable. Analysing butters with more than triplicate samples would improve the accuracy of the test but would make it cumbersome and inconvenient for routine analysis. In this project the Rutgers

Method on both whole butters and aqueous extracts as well as the AOAC Method applied to aqueous extacts were used to confirm results.

One of the goals of this project was to evaluate the present AOAC standard of acceptable AP activities in butter. The present standard is 2 units which is a value based on limited research done in the 1930's and 1940's. Because none of the in-house butters made from properly pasteurized creams exceeded this value no judgement can be made on its validity. This issue will be discussed in the section of this report covering the results of the commercial butters.

3.Microbial AP: No sample tested positive for microbial AP. This observation is in agreement with the typically low plate counts of the majority of the butters samples. The applicability of the present recommended method for detecting microbial AP in butter however, warrants further research. The use of this method has not been cited in most of the literature on AP activities in butter. Typically, it is concluded that low plate counts correlate with no microbial AP interference. Work should be initiated addressing the specific levels of a microbe's presence at which interfering AP is noticeable, the types of microbes which produce AP, and the characteristics (i.e. heat lability) of these microbial AP's if the present method for detecting microbial AP in butter will continue to be used. Such research is especially advisable in light of the recent work done by

Pratt-Lowe et al. (J. Dairy Sci. 71:17-23) which revealed that certain microorganisms produce heat labile AP.

4.Reactivated AP: The tests for reactivated AP in all samples were negative. However, activities in 5 of the 6 in-house butters increased during storage. Since these increases were not attributable to microbial AP some form of reactivation must be involved. This observation is not in agreement with the negative results of the AOAC Method for determining reactivated AP. The following comments address this issue.

The AOAC Method for detecting reactivated AP in butter is the same, unmodified method used for fluid milk products. No separate work has been discovered in literature applying its use for butter. Still, according to the AOAC Methods of Analysis the method is applicable and valid for butters.

Upon reviewing the protocol for reactivated AP determination it becomes evident that the unmodified procedure is difficult to carry out in analysis of butter for the following reasons (Figure 7):

1) The concentration of the magnesium solution is not altered for butter analysis. The described stock solution will result in an appropriate magnesium concentration when mixed with fluid milk products. The resulting concentration in an assay with butter however is much greater because butter is at least 80% fat.

2) Sample sizes in the AOAC procedure are given in units of volume (mls.). In working with butter units of weight (grams) were assumed.

3) The preincubation temperature in the AOAC protocol is 34C. At this temperature a sample and an aliquot of magnesium solution are mixed and held for one hour. Butter is not very fluid at 34C and therefore our analyses were done at 37C. At 37C proper mixing of sample and magnesium solution could be achieved.

4) Avoiding prewarming samples is recommended. How prewarming a sample affects the test is not explained. In working with butter prewarming is a necessary step before the magnesium solution of the test can incorporated.

5) Use of the AOAC procedure on butter is not evident in literature.

Thus, although an AOAC method for distinguishing AP activity in butter exists, its validity is questioned.

5.Aqueous Extract Analysis (AOAC Method): The use of the AOAC procedure for butter on aqueous extracts of butter samples proved to be a useful control for the analysis of the in-house butters. It was observed that the activities in the aqueous phases were about 10-15 times the activity of their respective whole butters. Therefore, low or negative activities in a butter can be confirmed by testing its aqueous extract. A butter with no activity will also have no activity in its aqueous extract. A sample with slight activity (1-2 units) will have an activity of perhaps 10-20 units in its aqueous phase.

Using aqueous phase analysis it was confirmed that the butter from vat pasteurized cream did indeed have no activity while the slight activities of the 185F and 195F HTST cream

FIGURE 7.

16.128 Residual Phosphatase (27)—Official Final Action

See **16.118.** Take sample from beneath surface with clean knife or spatula and proceed as follows:

Step 1.—Weigh 1.0 g sample (preferably in duplicate) on piece of waxed paper ca 2.5 cm sq and insert paper with sample into tube. Similarly, weigh another sample and place in tube as control or blank.

Step 2.—Heat *blank* ca 1 min to 85–90° in beaker of boiling H_2O (covered so entire tube is heated to 85–90°), and cool to room temp. From this point treat blank and test alike.

Step 3.—Add 10.0 mL buffer substrate prepd as in **16.116(b)**, except dissolve $Na_2C_6H_5PO_4$ in 100 mL undild Ba borate-hydroxide buffer made from 18 g $Ba(OH)_2.8H_2O$ and 8 g H_3BO_3/L. Stopper tube and mix.

Step 4.—Immediately after adding substrate, incubate 1 hr in H_2O bath at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min, heating to 85–90° (use thermometer in another tube of same size and shape contg same vol. of liq.), and cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1 mL 6.0% $ZnSO_4.7H_2O$ soln, and mix thoroly (pH of mixt. should be 9.0–9.1).

Step 7.—Filter (5 cm funnel, 9 cm Whatman No. 42 or No. 2 paper recommended), and collect 5.0 mL filtrate in tube, preferably graduated at 5.0 and 10.0 mL.

Steps 8—11.—Proceed as in **16.118.**

Step 12.—When using 1.0 g butter and adding 11.0 mL liq., multiply value of reading by 1.1 to convert result to phenol equivs/0.5 g butter. (Values >2 equivs/0.5 g indicate underpasteurization.)

See **16.129–16.130** for differential test for reactivated and residual phosphatase.

Reactivated and Residual Phosphatase, Differential Test (32)—Official First Action

16.129 ***Reagent***

Magnesium acetate soln.—40.1 g Mg/mL. Dissolve 35.4 g $Mg(OAc)_2.4H_2O$ in 25 mL H_2O, warming slightly. Transfer quant. to 100 mL vol. flask with H_2O, cool, and dil. to 100 mL.

16.130 ***Reactivation***

Place 10 mL of each sample in boiling H_2O bath and hold 1 min after temp. of sample reaches 95°. Cool, and use portion of each for diln, as required, and for boiled control. Place 5 mL aliquot of sample in screw-cap (phenol-free) test tube. Add 0.1 mL H_2O. To second 5 mL aliquot in identical tube add 0.1 mL $Mg(OAc)_2$ soln. Incubate both tubes 1 hr at 34°. Remove samples from bath and cool in ice-H_2O bath. Dil. aliquot of 1 mL sample contg Mg with 5 mL of corresponding boiled control. Test undild sample contg no Mg and 1+5 diln contg Mg for phosphatase activity as in **16.118, 16.123, 16.126,** or **16.164,** keeping tubes cool until analysis.

If 1 + 5 diln contg Mg has equal or greater phosphatase activity than undild sample contg no Mg, sample is regarded as neg. for residual phosphatase and indicates that phosphatase originally measured is of reactivated origin. If dild sample contains less activity than undild sample, it is considered pos. for residual phosphatase provided that initial conventional phosphatase test was pos. False pos. test for residual phosphatase may be obtained if reactivatable sample stood at elevated temp. (21–24°; 70–75°F) for ≥2 hr.

butters were due to the presence of AP and not because of error in measurements.

6.AP Activity in Butter (Rutgers Method): The Rutgers Method is a quick semi-quantitative test for AP analysis of milk. It was used in this project as an additional method to confirm the AOAC results. The results of the butters from underpasteurized creams were easy to interpret. The development of bright pink colors indicative of high activities greater than the 5 std. were obvious. The interpretation of the results from butters of properly pasteurized creams was more difficult. The slight presence of any pink color in these no or low activity samples was hard to detect within the opaque yellow color of the butters.

7.Aqueous Extract Analysis (Rutgers Method): Performing the Rutgers Method on aqueous extracts of butters eliminates the problem of color masking. Any pink color that develops is easily recognized in a white aqueous extract.

The Rutgers Method on aqueous extracts was used on the 12, 15, and 18 month samples. Relative activities were consistent with results of other methods,however they do not represent a 10-15 fold increase over whole butter activities as was observed with the AOAC Method on aqueous extracts. This is because while in the AOAC based Method 1 ml. of sample is diluted with 10 mls. of buffered substrate in the Rutgers Method the sample is incubated with just 1 drop of buffered substrate. In such an undiluted aqueous extract the salt and proteins are very

concentrated and this could affect the activity of AP. Future use of the Rutgers Method on aqueous extracts will require that specific assay conditions of sample size, substrate concentration, pH, dilution ranges, and incubation time be established.

8.AP Analysis (Dialysis Method): The Rutgers Method for AP determinations has the advantages of being fast and convenient. Also, phenolic compounds cannot interfere with this test as they can with the AOAC Method. A drawback of the Rutgers Method is the inability to quantitate outside the activities represented by the 3 available visual standards. The use of a dialysis procedure employing Rutgers Method reagents along with spectrophotometric analysis has been explored to develop a continuous quantitative assay procedure for AP analysis of butter. Results of initial experiments were given in Progress Report #3. Further work will be needed to establish specific assay conditions for the analysis of AP in butter using this method.

D. SUMMARY AND CONCLUSIONS

Six butters were manufactured and analysed for AP activity over 18 months of storage at 40F, 0F, and -20F. The samples included 3 butters made from properly pasteurized creams and 3 made from under or unpasteurized creams. Activities in the butters from the first group never reached 2 units, the present AOAC value of acceptance. Activities in the samples of the second group ranged from about 100 units (mixed cream butter) to over 500 units (raw cream butter). Storage temperature did not significantly affect AP activity in any of the butters.

Increases in AP activitiy were observed in all butters except the one from vat pasteurized cream. The increases for the properly pasteurized HTST cream butters were very slight, perhaps because they were batch and not continuously churned. Activities in the under and unpasteurized butters doubled after 12 months of storage. These relatively great increases were unexpected and unexplainable. It would be interesting to explore the reason behind this doubling of AP activity in these butters made from creams of little heat treatment.

None of the activity observed in the in-house butters were attributable to reactivated or microbial AP. Aqueous phase analysis as well as testing using the Rutgers Method proved helpful in confirming results obtained by the AOAC Method. The development of these unofficial methods for AP analysis of butter should be further explored.

III. COMMERCIAL BUTTERS

A. Introduction

B. Results

C. Discussion

D. Summary and Conclusions

A.INTRODUCTION

Thirty-seven butter samples and their pasteurization parameters were supplied by the USDA. AP activities of the whole butters and their aqueous extracts were determined as well as salt contents of the aqueous phases. The results were given in Progress Report #5 and are repeated in Table 1. The following is a summary and discussion of the results.

B.RESULTS:

1.Vat Pasteurized: Only 1 butter was made from vat pasteurized cream. It had no activity. This is consistent with typically observed AP activities of butter made from vat pasteurized cream.

2.Unsalted Butters: Four of the samples were determined to be unsalted. These had no activity in either the whole butters or their aqueous extracts.

3.AP Negative Samples: Eighteen of the 37 butters had less than 2 units of activity. These include salted butters, unsalted butters, and butters of undetermined salt content.

4.AP Positive Samples: Nineteen of the 37 butters had greater than 2 units of activity. Activities were confirmed by Rutgers Method and aqueous extract AP determinations. Seventeen of these samples were salted while 2 had unknown salt contents.

Table 1. AP Activities on Butters Supplied by USDA

SAMPLE (Plant #)	PAST METHOD (Temp°F/Time)	SALT CONTENT (% of aq phase)	AVG AOAC AP ACTIVITY Butter[1]	Aq Extract[2]
1. Commercial Cry Spokane	170/30 min	NA	-.13	-.13
2. Land O'Lakes	187/19 sec	0	-.19	-.19
3. 47-330	190/22 sec	0	-.13	.06
4. 06-06 (See #15)	182.5/29.7 sec 182.5/27.5 sec	0	.06	1.29
5. 55-304 (See #21)	186/35 sec	0	.45	-.06
6. 31-212	185.5/48.7 sec 186.5/45.6 sec	8.21	-.17	3.43
7. 55-292	189/41.6 sec	7.14	0	-1.04
8. 40-70	188/19.4 sec	6.19	.13	.53
9. Knudsen/Tipton	184.0/26.1 sec	NA	.26	19.0
10. 29-534	186/17.16 sec	NA	.32	NA
11. CACOOP/Petaluma	186/19.5 sec 183/19.5 sec	NA	.39	6.0
12. SJVD/Los Banos (see #19, #25)	188/44.0 sec 181.25/44.0 sec 180.5/48 sec	NA	.45	27.0
13. 36-1548	193/20.5 sec 186/20.5 sec	NA	.71(M^-,R^-)	10.5
14. 55-376	185/70 sec	7.35	.94	13.46
15. DCCA/Tulare (See #4)	185/27.5 sec 184/27.5 sec	NA	1.16(M^-,R^-)	16.0
16. 48-1010	192.5/35.3 sec 186/35.3 sec	7.88	1.85	23.75
17. 53-48 (See #32)	185/30.1 sec 184/22.0 sec	5.14	1.88	25.88

Continued on page two

Table 1. AP Activities on Butters Supplied by USDA - continued

SAMPLE (Plant #)	PAST METHOD (Temp°F/Time)	SALT CONTENT (% of aq phase)	AVG AOAC AP ACTIVITY Butter[1]	Aq Extract[2]
18. Crystal/Sacto	188/24.0 sec 183/24.0 sec	NA	1.96(M^-,R^-)	23.0
19. 06-748 (See #12,#25)	W 181 E 181	6.50	2.01	23.75
20. 31-67	187/25 sec 185/16.6 sec	6.93	2.20	31.58
21. 55-304 (See #5)	186/35 sec	4.37	2.46	50.49
22. 27-484	188/32.98 sec 185/17.34 sec	6.21	2.52	26.72
23. Humbolt/Fortuna (See #33)	191/27.0 sec 186.5/27.0 sec	NA	2.72(M^-,R^-)	48.0
24. 20-350	186/17.16 sec	9.55	3.30	34.23
25. 06-748 (See #12, #19)	181.75/49 sec 182/48 sec	4.47	3.66	34.00
26. 55-653	186.5/27 sec	6.99	3.82	27.43
27. 4-30	187.5/23.2 sec	7.55	4.44	35.07
28. 48-908	190/34 sec 186/34 sec	7.24	4.53	42.38
29. 55-360	188.5/40.9 sec 187/39.1 sec	7.60	4.94	44.88
30. Land O'Lakes/ Mtn. Lake, MN	190/35 sec 187/35 sec	NA	5.82(M^-,R^-)	87+
31. 49-4	189/49 sec 185/49 sec	6.78	5.99	52.48
32. 53-48 (See #17)	185/30.1 sec 184/22.0 sec	7.25	6.60	69.82
33. 06-1187 (See #23)	185.5/30 sec	8.33	6.75	72.28
34. 41-25	195/27.5 sec 165/27.5 sec	5.38	7.70	76.18

Continued on page three

Table 1. AP Activities on Butters Supplied by USDA - continued

SAMPLE (Plant #)	PAST METHOD (Temp°F/Time)	SALT CONTENT (% of aq phase)	AVG AOAC AP ACTIVITY Butter[1]	Aq Extract
35. 29-424	187/23.1 sec	8.56	7.96	85+
36. 36-5056 (See #36)	195/36.8 sec 186/31 sec	5.84	15.72	113.75
37. 36-5056 (See #36)	195/26.8 sec 187/26.8 sec	6.32	16.40(M^-,R^-)	137.28

1. Units are in µg phenol/0.5 g butter/1 hour.

2. Units are in µg phenol/0.5 ml aqueous extract/1 hour

$M^+/^-$: Microbial AP; positive/negative

$R^+/^-$: Reactivated AP; positive/negative

NA: Not available

5.Microbial and Reactivated AP: Eight butters were tested for microbial and reactivated AP. All of these samples tested negative for both forms of AP.

C.DISCUSSION

1.Effect of Pasteurization Temp. on AP Activity: No direct correlation could be made between pasteurization temperature and AP activity. This is because of the different holding times used by the manufacturers. Still, it can be observed that butters #36 and #37 have about twice the activity of any other sample. These are salted butters made from 195F HTST creams. A third 195F HTST cream also had a high AP activity. This reflects the common observation associating a high degree of AP reactivation with high pasteurization temperatures.

2.AOAC Method: The present AOAC procedure proved to be sensitive in detecting AP activities in butter. A wide range of activities were detected in these butters and these activities were confirmed by Rutgers Method and aqueous phase analysis.

The present standard of 2 units appears to need modification. Over 50% of the commercial butters had positive activities based on this value and therefore should be considered to be made from underpasteurized or contaminated creams. This is highly unlikely. Perhaps the standard is not applicable to today's butters made in continuous churns from HTST creams.

3.Microbial and Reactivated AP: As was the case with the in-house butters tests for microbial and reactivated AP resulted negative. The applicability of these tests to butter is questioned.

4.Aqueous Extract AP Analysis: Rutgers and AOAC based aqueous extract AP determinations were used to confirm the measured activities of the whole butters. These analyses were very useful for samples with low whole butter activities. Based on the aqueous phase activities butters with low activities can be distinguished from butters with no activity. Aqueous extract analysis was less useful for butters with high activities.

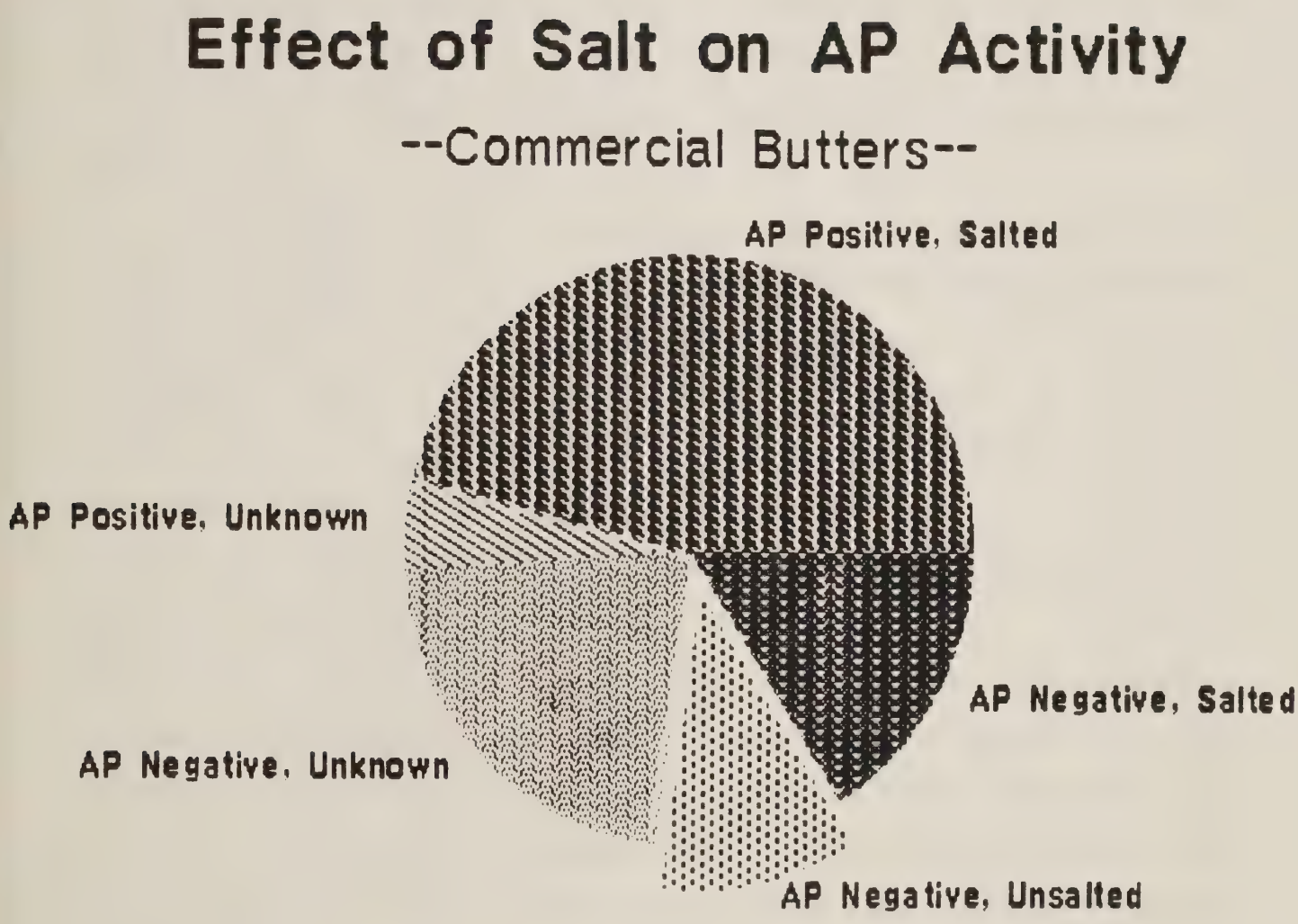
5.Effect of Salt on AP Activity: The effect of salt on AP activity in butter was considered. The pie chart (Figure 8) separates the 37 commercial butters on basis of AP activity (pos.or neg.) and salt content (salted, unsalted, or unknown salt content). The 2 upper sections of this chart represent the AP positive samples. It is evident that a majority of these butters are salted (larger section). No confirmed unsalted butters were AP positive. The lower 3 sections represent the AP negative butters. Included in this group are salted butters, butters of unknown salt content and the only unsalted butters. Thus, it appears that the presence of salt does affect AP activity. To confirm this a follow-up study was done.

[illegible] tests [illegible] for commercial [illegible] results [illegible]. The applicability of [illegible]

[illegible] AP [illegible] Determinations were used to confirm the reason [illegible] of the whole butters. These [illegible] samples with low whole butter activity [illegible] phase activities [illegible] from butters with [illegible] activity [illegible] was less useful for butters with high activities.

Effect of Salt on AP Activity. The effect [illegible] activity in butter was considered. The pie chart (Figure 8) [illegible] the 37 commercial butters on basis [illegible] activity [illegible] or neg.) and salt content (salted, unsalted, or unknown salt [illegible]). The 3 upper sections of this chart represent the [illegible] samples. It is evident that a majority of these [illegible] (larger section). No confirmed unsalted [illegible] The lower 3 sections represent the AP negative [illegible] are salted butters, [illegible] unsalted butters. Thus, it [illegible] does alter AP activity. To [illegible] was done.

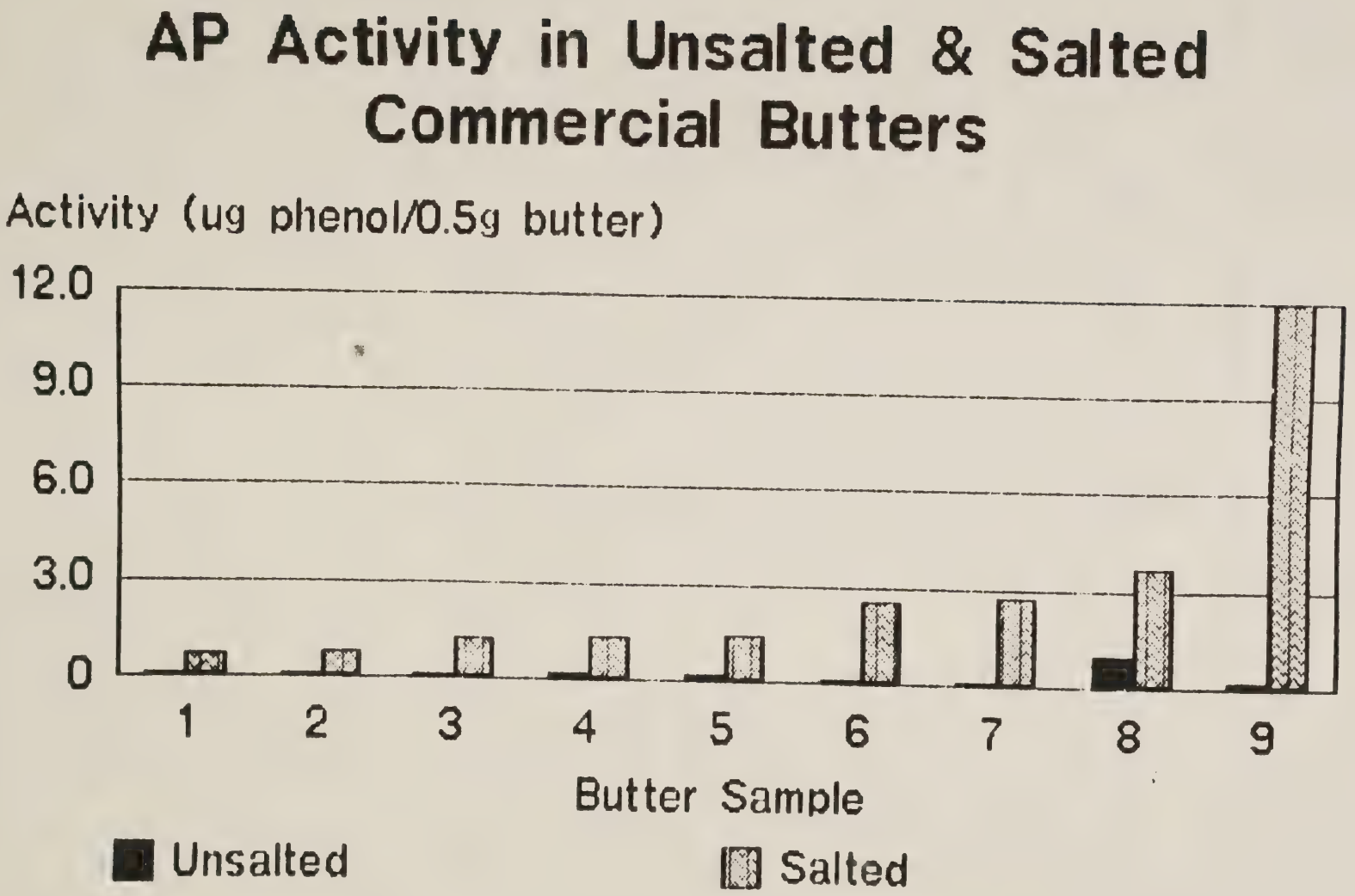
FIGURE 8.


Effect of Salt on AP Activity
--Commercial Butters--
AP Positive, Salted
AP Positive, Unknown
AP Negative, Salted
AP Negative, Unknown
AP Negative, Unsalted

Nine brands of salted and unsalted butters were obtained and analyzed for AP activity. Results appear in Figure 9. It is evident that the salted version of each brand had noticeably greater activity than its unsalted counterpart. Four of the salted butters had over 2 units of activity. All the unsalted butters had virtually no activity. The activity of the unsalted #8 was .91 units.

It appears that the presence of salt does affect AP activity in butter and in fact it is the salted butters that have accounted for the AP positive samples in both the in-house and commercial butter studies. Just how salt affects AP activity will have to be a subject for future research. Perhaps salt is involved in influencing reconformation of a reversibly inactivated AP or maybe there is an interaction between salt and the fat globule membranes causing the release of AP from a previously protective structure. Whatever the reason, understanding the relationship between salt content and AP activity could shed light on the mechanism of AP reactivation as well as provide useful information on various related issues such as enzyme stabilization/inactivation or enzyme-membrane interactions.

FIGURE 9.


AP Activity in Unsalted & Salted Commercial Butters
Activity (ug phenol/0.5g butter)
12.0
9.0
6.0
3.0
0
1
2
3
4
5
6
7
8
9
Butter Sample
Unsalted
Salted

D. SUMMARY AND CONCLUSIONS

Thirty-seven commercial butters and their pasteurization parameters were supplied by the USDA for AP analysis. These samples represented a wide range of manufacturing methods. The results of these samples were a useful supplement to those of the in-house butters in giving a better understanding of AP activity in stored butters.

Over 50% of the commercial butters had positive AP activities. These activities were not attributable to either reactivated or microbial AP based on present official methods. The presence of salt was implicated as a possible factor influencing the high activities observed. More work is recommended to pursue the effect of salt on AP activity in butter.

The AOAC Method for determining residual activity was adequate in detecting a wide range of activities. The present 2 unit standard however needs to be reevaluated. Testing AP by aqueous phase analysis and by using Rutgers Method based tests were useful in confirming results by the AOAC Method. Future use of these unofficial methods for AP analysis of butter will require additional work.

IV. PROPOSED FUTURE RESEARCH

Based on the shelf life study on the in-house butters and AP analysis of commercial butters several areas of future study have been identified. These include investigating traditional as well as newer approaches of AP analysis; a few are listed below:

1) Reevaluation of the present 2 unit standard for butter.
2) Reevaluation of the use and interpretation of the present methods for microbial and reactivated AP analysis.
3) Development of aqueous phase AP analysis methods.
4) Development of Rutgers Method based tests for AP analysis.

Also, the use of different biochemical and chemical methods for AP analysis should be considered as should the subject of the effect of salt on AP activity. Eventually the mechanisms of reactivation need to be elucidated to confirm the validity of AP analysis as a tool for controlling pasteurization of dairy foods today.

V. ADDENDUM

A. Introduction

B. Results

C. Discussion

D. Conclusions

A. INTRODUCTION

It was noticed that the presence of salt could influence AP activity in butter. This observation was based on analyses on two sets of commercial butters. Of 37 samples supplied by the USDA 4 were confirmed unsalted. None of these 4 had AP activity in either the whole butter or aqueous phase. A subsequent investigation was done where 9 brands of salted and unsalted butters were tested and compared for AP activity. In all 9 brands the salted butter had noticeably more activity than the unsalted counterpart, which had virtually no activity.

The above experiments include analysis of a total of 13 unsalted samples. To get a better understanding of the effect of salt on AP activity additional unsalted butters were supplied by the USDA. The following supplement to the final report addresses the results of these final samples.

B. RESULTS

1.General: Sixteen additional butter samples were supplied by the USDA.Samples were tested for AP activity in both the whole butter and aqueous phase. Salt content analysis was performed on the aqueous phase. Results of the analyses are given in Table 2. Also included in the table are the cream pasteurization parameters of the respective butters as well as the plants of manufacture. Multiple samples were received from four different butter manufacturers resulting in the 16 total samples.

2.Salt: Salt tests performed on aqueous extracts confirmed that all butters were unsalted.

3.AP Negative Butters: Twelve of the 16 butters had no activity in either the whole butter or aqueous phase analyses. This is consistent with a hypothesis that the presence of salt positively affects AP activity whereas in the absense of salt activities are minimal.

4.AP Positive Butters: Four of the 16 butters had greater than 2 units of activity. High aqueous phase activities confirmed the results. All of these butters were produced by the same plant. The high activities are inconsistent with the hypothesis previously mentioned. It is interesting to note that all of these 4 AP positive butters were manufactured at the same plant.

Table 2. AP Activities in Unsalted Butters

SAMPLE (Plant #)	PAST METHOD (Temp°F/Time)	SALT CONTENT (% of aq phase)	AVG AOAC AP ACTIVITY Butter[1]	Aq Extract[2]
35-5056	195/29.7 sec			
1183		0	-.13	-.13
1184		0	-.19	.19
1185		0	-.13	-1.16
1186		0	-.06	.58
1187		0	0	-.06
55-304	186/35 sec			
82-17		0	.13	1.55
82-18		0	.06	0
82-19		0	0	.19
27-31	193/34 sec			
A		0	-.71	-1.10
B		0	.06	-.52
C		0	-.32	-.52
D		0	-.97	-.13
20-350	186/17.16 sec			
1		0	3.59	33.78
2		0	5.27	41.61
3		0	2.85	26.01
4		0	2.39	23.10

1. Units are in µg phenol/o.5 g butter/1 hour.

2. Units are in µg phenol/0.5 ml aqueous extract/1 hour.

C. DISCUSSION

Based on results from previous experiments negative activities were expected in these 16 unsalted butters. A majority (12 of 16) of the samples did indeed have negative activities. However, the fact that 4 butters had relatively high activities is puzzling and counter to what was expected. This puts to question the issue of salt influencing AP activity in butter and would seem to indicate that more work is needed in this area. A few observations are noted below.

A total of 9 of the AP positive butters were supplied by 2 plants using pasteurization temperatures greater than 190F. It has been previously noted that using such high temperatures usually results in butters with high AP activities. These samples however, had no activity in either the whole butter or aqueous phase. One explanation of the lack of activity could be the absence of salt in these samples. Other factors such as method of churning (continuous vs. batch) could also have influenced AP activity. Still, the possibility that salt can affect AP activity in butter remains very plausible.

Eight of the 16 butters were made from 186F treated creams. Four of these had no activity in either the whole butters or the aqueous extracts. The holding time of these four samples was 35 seconds. The remaining 4 butters were made from 186F/17.16sec. cream. High activities were evident in both whole butters and their aqueous extracts.

These final observations do not support a clear-cut relationship between AP activity and the presence of salt.More specifically, 4 of the total 16 unsalted samples had definite positive activities. The premise had been that unsalted butters tend to have very little or no activity. Perhaps there are other reasons accounting for the high activities in these 4 butters. If such other factors exist the relationship between salt and AP activity may still be a valid hypothesis.

Since the 4 AP positive samples were all made at the same plant it would be logical to assume that the manufacturing procedure at this plant were in some way significantly different than the process at the other plants. It would be interesting to find out if any such differences did exist. Perhaps a unique churning or cream handling procedure was used. An obvious aspect to consider is the cream pasteurization itself. The 186F/17.16 second heat treatment used is a legal pasteurization but it represents one of the lowest temperatures and shortest holding times used of any of the samples studied in this project. It would not be unreasonable to suspect that this relatively low heat treatment does not totally inactivate AP.

D. CONCLUSIONS

In conclusion it seems that salt may have an affect on AP activity in butter. Unsalted butters tend to have lower activities than salted butters made under similar conditions. It would be interesting to find out why unsalted samples from plant #20-350 had unusually high activities. Although the relatively low albeit legal pasteurization used at this plant could be the reason for the high activities it would be worth the effort to consider other aspects of the butter manufacture used at this one plant.